

Isolation and identification of 2,4,5-trimethyl-3-oxazoline and 3,5-dimethyl-1,2,4-trithiolane the volatile flavour compounds of boiled beef*,†

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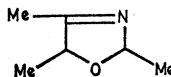
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Several attempts have been made by previous researchers to identify the volatile compounds which are responsible for the aroma of boiled beef.¹⁻⁶ However, the few common simple carbonyls, mercaptans, sulphides, and ammonia identified represent neither the complete spectrum of volatile flavour compounds in boiled beef nor the crucial compounds with characteristic boiled beef aroma.

Recently, we isolated the volatile compounds from 56 lb of semitendinosus muscles of Hereford steers from the same feed lot. The beef was boiled in water and the volatile flavour compounds were then isolated by a specially designed apparatus utilising the principles of flash vaporisation and evaporation from a continuous thin film heated under vacuum.⁷ Preparative gas chromatography of the isolated volatile compounds yielded 18 broad fractions. Two of these fractions had characteristic boiled beef aroma. Rechromatography of these two broad fractions yielded two novel cyclic nitrogen and sulphur compounds - 2,4,5-trimethyl-3-oxazoline from one fraction and 3,5-dimethyl-2,4-trithiolane from the other.

The gas chromatographic fraction identified as 2,4,5-trimethyl-3-oxazoline,



had a retention time of 9 min 9 sec on a $\frac{1}{8}$ in \times 10 ft column packed with 15 per cent Carbowax 20M on 70/80 mesh Anakrom ABS at a temperature programmed from 50-200°C at 6°C per min with a helium flow rate of 60 ml. per min. Its infrared and mass spectra are shown in Fig. 1. The identification was confirmed by the infrared and mass spectra and retention time of the authentic compound which was synthesised according to the procedure of Jassmann and Schultz.⁸ The reaction products of aqueous ammonia, acetaldehyde, and acetoin were extracted with ethyl ether. After the extract was freed of solvent, it was fractionally distilled and the fraction collected at 45-47°C under 12 mm.Hg. was finally purified by gas chromatography.

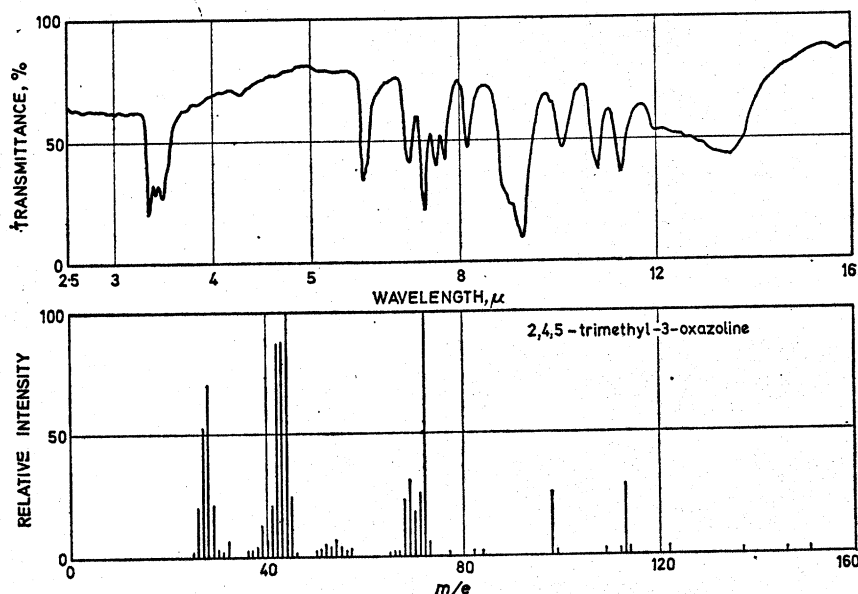


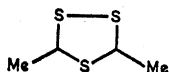
Fig. 1 Infrared and mass spectra of the gas chromatographic fraction identified as 2,4,5-trimethyl-3-oxazoline

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The infrared and mass spectra of other gas chromatographic fractions of the volatile flavour compounds isolated from the boiled beef indicated that homologues of 2,4,5-trimethyl-3-oxazoline were also present, particularly that with an alkyl group of mass 55.

The gas chromatographic fraction identified as 3,5-dimethyl-1,2,4-trithiolane



had a retention time of 17 min 6 sec on a $\frac{1}{8}$ in \times 20 ft column packed with 15 per cent Carbowax 20M on 70/80

isomers of 3,5-dimethyl-1,2,4-trithiolane were present.

The presence of gas chromatographic fractions with characteristic boiled beef aroma and the identification of some novel cyclic nitrogen and sulphur compounds in the volatile fraction of boiled beef indicated the possibility that the characteristic boiled beef flavour is predominantly due to some crucial compounds.

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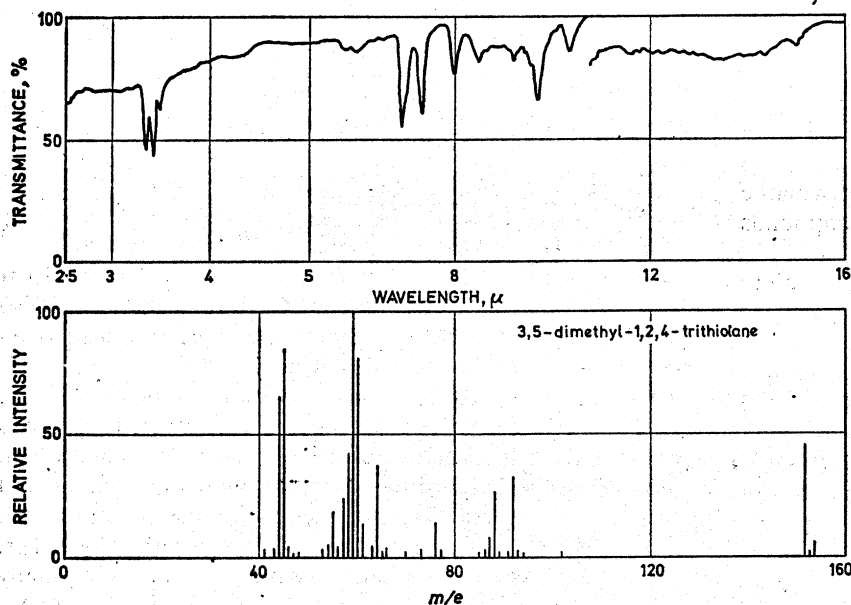


Fig. 2 Infrared and mass spectra of the gas chromatographic fraction identified as 3,5-dimethyl-1,2,4-trithiolane

mesh Anakrom ABS at a temperature programmed from 50–200°C at 6°C per min with helium flow rate of 40 ml. per min. Its infrared and mass spectra are shown in Fig. 2. The identification was confirmed by the infrared and mass spectra and the retention time of the authentic compound which was synthesised according to the procedure of Asinger *et al.*⁹ by the reaction of acetaldehyde, sulphur and hydrogen sulphide in the presence of diisobutylamine. The reaction product was then purified by gas chromatography.

The infrared and mass spectra of another gas chromatographic fraction of the volatile flavour compounds isolated from boiled beef suggested that both the *cis* and *trans*

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